Responses of root architecture and the rhizosphere microbiome assembly of maize (*Zea mays* L.) to a soil texture gradient

Lioba Rüger[1], Kai Feng[2,3], Yan Chen[4], Ruibo Sun[5], Bo Sun[4], Ye Deng[2,3], Doris Vetterlein[6,7], Michael Bonkowski[1]

[1]Terrestrial Ecology, Institute of Zoology, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, Zülpicher Str 47b, 50674 Köln, Germany

[2]CAS Key Laboratory for Environmental Biotechnology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China

[3]College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, China

[4]Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China

[5]Anhui Province Key Laboratory of Farmland Ecological Conservation and Pollution Prevention, Key Laboratory of JiangHuai Arable Land Resources Protection and Eco-Restoration, College of Resources and Environment, Anhui Agricultural University, Hefei, China

[6]Department of Soil System Science, Helmholtz Centre for Environmental Research – UFZ, Halle, Germany

[7]Soil Science, Martin-Luther-University Halle-Wittenberg, Halle, Germany

Corresponding authors:

Michael Bonkowski

M.Bonkowski@uni-koeln.de

ORCID: 0000-0003-2656-1183

Lioba Rüger

lioba.rueger@uni-koeln.de

ORCID: 0000-0001-7168-0005

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Abstract

Soil texture, i.e. the fractions of different sized mineral particles, is critical to root growth and an important determinant of the occurrence and distribution of soil microbiota. More recently it was shown that individual plant species and even different cultivars harbor highly distinct rhizosphere associated microbiota, but it is still an open question how soil texture and its influence on root growth feeds back on root microbial assembly.

We manipulated soil texture by stepwise additions of quartz sand to an agricultural loam. We grew maize (*Zea mays* L.) in these soils, measured changes in root traits and sampled bulk soil and rhizosphere to apply amplicon based high-throughput sequencing. We investigated changes in root morphology of maize and the concomitant shift in prokaryote (archaea and bacteria) and protist (Cercozoa and Endomyxa) diversity, community composition and co-occurrence in the maize rhizosphere along the soil texture gradient.

A linear relationship between loam fraction and root morphology and a shift in microbial diversity along the soil texture gradient, as well as a stronger selection effect of the rhizosphere in soils with a high sand fraction (and high bulk density) were found. Co-occurrence network analysis revealed high modularity in fine textured soil, demonstrating that bulk density and texture are important factors affecting the recruitment of the core rhizosphere microbiome of maize.

Keywords: rhizosphere microbiome, protists, prokaryotes, root architecture, soil texture

1. Introduction

Soil texture, i.e. the fractions of different sized mineral particles, affects soil porosity and aggregation, thereby influencing parameters of soil strength and consequently the soil mechanical impedance to root elongation (Rich and Watt, 2013). Increased mechanical impedance was generally found to slow down root elongation rates while root diameters increase (Bengough and Mullins, 1990; Zou et al., 2001; Potocka and Szymanowska-Pułka, 2018; Vanhees et al., 2021), and this may feed back on the size and the architecture of the whole root systems (Barley and Greacen, 1967; Laboski et al., 1998; Grzesiak, 2009). At the same time, soil texture determines the physical habitat of microorganisms, such as soil surface area, porosity, aggregation and consequently matric potential, gas exchange rates, diffusion rates of nutrients and substrates, as well as habitable pore space (Elliott *et al.*, 1980; Chenu & Stotzky, 2002; Or *et al.*, 2007; Holden, 2011). Thereby soil texture exerts a strong habitat filtering effect on microbial community composition (Chau et al. 2011; Neumann et al. 2013; Vos et al. 2013; Ebrahimi and Or 2014; Hu et al. 2014; Florentin et al. 2015; Naveed et al. 2016).

Generally, coarser grained soil (e.g. sand) is associated with a larger share of macropores, increasing gas diffusion and decreasing water holding capacity. In sandy soils, restricted water films may entrap

microbial cells and lead to higher bacterial species richness (Chau et al. 2011). On the other hand, Sessitsch et al. (2001) reported comparably low bacterial diversity and biomass in coarse-textured soils and assumed a relation with low nutrient availability, protist grazing and competition with fungal organisms.

In fine textured soils the decreased overall pore connectivity restrict the movement and dispersal of bacteria (Ebrahimi and Or, 2014), thereby increasing the small-scale heterogeneity of communities (Vos et al., 2013; Szoboszlay and Tebbe, 2021). Fine textured soils were found to favor bacteria with specific traits, like filamentous bacteria (Xia et al. 2020) or tolerance to anaerobic conditions due to restricted gas exchange (Or et al., 2007; Carminati and Vetterlein, 2013). In addition, bacterial survival is much improved by physical protection in aggregates and a narrow, tortuous pore system as found in fine textured soils (Marshall 1975; Heijnen et al. 1993). For example, most of the ubiquitous cercozoan and endomyxan protists have cell diameters of 10 to 50 µm, whereas their bacterial prey (≤ 0.5 µm diameter) is one to two orders of magnitude smaller (Bae et al. 1972, Christensen et al. 1999, Dumack et al. 2019). Even though flexible bodies and slender pseudopods of protists allow predation in narrow crevices (Hattori et al. 1994), small pores act as predator-free refuges and protect prokaryotes from predation by protists (Elliott et al., 1980; Darbyshire et al., 1985; Heynen et al., 1988; Rutherford and Juma, 1992; England et al., 1993; Hassink et al., 1993; Wang et al., 2005). Generally loamy soils contain higher amounts of organic carbon compared to soils with higher sand fractions. The higher availability of carbon as a nutrition source for soil microbiota might further affect their abundance and diversity.

Both roots and microorganisms actively modify their physico-chemical environments (Watt et al. 1993). The mechanical force of root growth can locally enhance soil compaction (Vollsnes et al. 2010; Phalempin et al. 2021), and roots affect the soil hydraulic conductivity through rhizodeposition (Angers and Caron, 1998; Bodner et al., 2014; Benard et al., 2019), and by favoring microbial exopolymer production (Watt et al. 1993; Watt et al. 1994; Chenu and Cosentino 2011, Landl et al. 2021). These processes are highly dynamic in time and space. Maize roots for example were shown to proliferate between 4 to 8 cm day^{-1} in soil (Iijima et al., 2003), while the daily rate of rhizodeposition was estimated to be 6 times that of root production (Molina et al., 2001). The root tip initiates rhizosphere community assembly when these rhizodeposits activate bulk soil bacteria from their dormant state (Dupuy and Silk 2016; Bonkowski et al. 2021). But since rates of root proliferation depend on soil mechanical impedance (Iijima et al., 2000; Watt et al., 2006), soil texture may strongly affect patterns of rhizosphere microbiome assembly along the longitudinal root axis (Rüger et al., 2021). Consequently, the interactions between plant root systems and soil texture are complex, and little is yet known about how these interactions feed back on rhizosphere community structure. We manipulated soil texture

by diluting an agricultural loam soil at different proportions with pure quartz sand achieving mixtures with 20, 40, 60, 80 and 100% loam. We grew maize at different soil textures and analyzed changes in root architecture and the composition of the prokaryotic (bacteria and archaea) and protistan (Cercozoa and Endomyxa) microbiome. We hypothesized that the gradual changes in soil texture would cause gradual changes in root architecture, and that both together would further propagate in terms of community assembly and result in changes in community composition of rhizosphere microbiota. We further hypothesized that co-occurrence networks between prokaryotic and protistan microbiota will reflect the differences in soil texture due to differences in motility of predators and the accessibility of bacterial prey.

2. Material and Methods

2.1. Experimental set up

The soil substrates consisted of a loam and sand fraction. The loam soil was a Phaeozem that had been under agricultural use until it was excavated to a depth of 50 cm and thoroughly sieved (1 mm mesh size) for homogenization. The particle size fraction of loam was 33/48/19 for sand/slit/clay. The sand material was a pure quartz sand obtained from the depth of a quarry (Vetterlein et al., 2021). To achieve different soil textures the loam was mixed with quartz sand at five different proportions, containing 20, 40, 60, 80 and 100% of loam (Fig. 1). Thereby, the quartz sand was inoculated with a substantial microbial community originating from loam of the plough horizon of an agricultural field. Before sand and loam were mixed, both were fertilized according to Vetterlein et al. (2021) to compensate for differences in nutrient availability.

In the experiment, cylindrical persplex tubes (200 mm height, 70 mm inner diameter), filled with 885 cm^3 of the differently composed soil mixtures, served as microcosms. A nylon gauze (30 µm mesh size) was placed at bottoms of tubes to retain soil but enable watering. To ensure comparable compaction and porosity throughout all replicates of each soil mixture, soil was filled into tubes through a horizontally moving sieve (4 mm) and compacted by repeated stamping tubes on a flat surface. The final bulk density was determined by weighing each filled tube. In this manner 80 microcosms were prepared, 40 for the analysis of the soil microbiome and 40 additional ones for the analysis of root traits, resulting in eight replicates per soil texture treatment. The basic design was a one factorial randomized block design. Before microcosms were planted with *Zea mays* (inbred line B73), seeds were surface sterilized for 10 min in 10% H_2O_2 and placed on wet filter paper for gemination. After three days under sterile conditions at 18 °C in the dark, seedlings most similar in size were selected and transferred to the prepared microcosms. Throughout the experiment the volumetric water content was kept at a constant level of 22% by daily weighing and accordingly replacing water from the bottom and top of microcosm tubes. Soil and roots were protected from light by a layer of

aluminum foil around tubes. Microcosms were placed in a climate chamber with a day-night regime of 12/12 h (350M PAR) at 24 °C/18 °C and 65% humidity for nine days.

2.2. Sampling

For microbiome analysis root sections of 1 cm with adhering soil from (i) root hair zones, (ii) regions where earliest lateral roots had emerged, and (iii) from regions with fully developed lateral roots were transferred into sterile 15 ml centrifuge tubes. Samples from primary and two seminal roots of each plant were pooled per root region to form biological replicates. In the following, the three sampled root regions are summarized under the term 'rhizosphere' and are treated as replicates, resulting in 8x3 replicates for each treatment. As a control, five randomly chosen bulk soil samples were pooled per microcosm. To obtain rhizosphere soil for DNA extraction, roots were vortexed in a 0.3% NaCl solution. The solution was centrifuged for 30 min at 5000 x *g*, the supernatant discarded, and the remaining soil pellet used for DNA-extraction with the FastDNA® SPIN Kit for soil and subsequent purification with the GENECLEAN® SPIN Kit (MP Biomedicals, Santa Ana, CA, USA), following the manufacturer's instructions.

For measurements of root parameters whole root systems of spare replicates were rinsed with distilled water to clean them from adhering soil, fixed with Formalin-Acetic Acid, 1:1:18 vol% of 35% formaldehyde, glacial acetic acid, and 70% ethanol and stained with ink (Parker Quink). The whole root systems were scanned for total root length, length of axial and lateral roots, root surface area and root diameter using WinRHIZO (V5.0, Regent Instruments, Quebec, Canada). Maize shoots were dried (60 °C, 48h) and weighed.

2.3. Amplicon-sequencing and sequence processing of Cercozoa and Endomyxa

To amplify a circa 350 bp long fragment of the V4 region of the SSU/18S gene of Cercozoa and Endomyxa a two-step PCR was conducted with tagged group specific primers (Fiore-Donno et al. 2020). Briefly, the forward primers S615F_Cerco (5'- GTTAAAAAGCTCGTAGTTG -3') and S615F_Phyt (5'-GTTAAAARGCTCGTAGTCG -3') and the reverse primer S963R_Phyt (5'-CAACTTTCGTTCTTGATTAAA-3') were used in a first PCR. Subsequently a second, semi-nested PCR was performed for further amplification and sample indexing. This time the forward primer S615F_Cer (5'GTTAAAARGCTCGTAGTYG-3') and the reverse primer S947R_Cer (5'-AAGARGAYATCCTTGGTG-3'), both tagged with barcodes, were used. Concentrations of reagents and the thermal program used for PCRs were similar to those used in Rüger et al. (2021). To confirm successful amplification and to exclude potential contamination, PCR-products were checked by gel electrophoresis. With the SequalPrep Normalization Plate kit (Invitrogen GmbH, Karlsruhe, Germany) samples were purified and consistent concentrations were obtained. Finally, pooled amplicons were sequenced on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at the Cologne Center for Genomics (Cologne,

Germany) using the v3 Reagent kit. Performing 2x300 cycles, 300 bp long paired-end reads were produced.

Sequences were processed, following the pipeline described by Fiore-Donno et al. (2020). First, MOTHUR v. 39.5 (Schloss et al., 2009) was used to merge paired-end reads not allowing any mismatches in primer and barcode sequences, maximum two mismatches and one ambiguity in the target sequence and an minimum overlap of 200 bp. After demultiplexing and trimming of primer and tag sequences reads were clustered into operational taxonomic units (OTUs) using VSEARCH (Rognes et al., 2016) with the abundance-based greedy algorithm (agc) at a similarity threshold of 97%. To avoid misinterpretation of amplification or sequencing noise (Fiore-Donno et al., 2018), OTUs represented by less than 500 reads were excluded from further processing. The assignment of OTUs to taxa was conducted using BLAST+ (Camacho et al., 2009) with an e-value of 1^{-50} with the PR^2 database (Guillou et al., 2013) as reference. In MOTHUR sequences were aligned with a template provided by Fiore-Donno et al. (2018), allowing gaps of maximum five nucleotides and finally, chimeras were identified with UCHIME (Edgar et al., 2011) and removed.

2.4. Amplicon-sequencing and sequence processing of bacteria and archaea

The circa 250 bp long fragment of the V4 region of the SSU/16S genes of bacteria and archaea was done using the forward primer 515F (5'-GTGCCAGCMGCCGCGGTAA-3') (Caporaso et al., 2011) and the reverse primer 806R (5'-GGACTACNVGGGTWTCTAAT-3') (Apprill et al., 2015). Again, concentrations of reagents and the thermal program used for PCRs were described in Rüger et al. (2021). Amplicons were sequenced on an Illumina HiSeq platform (Illumina Inc., San Diego, CA, USA) by Magigene Technology Co., Ltd. (Guangzhou, China) using the HiSeq v2 Reagent kit. Performing 2x250 cycles, 250 bp long paired-end reads were produced.

Before processing sequences, low quality reads and adapter sequences were removed with Trimmomatic (Bolger, Lohse et al. 2014). Using fastq-join (Aronesty 2011) paired-end reads were merged when an overlap of 10 bp was detected, allowing 10% difference within the overlapping region and no errors within primer sequences. Thereafter, primer sequences were trimmed using cutadapt (Martin, 2011). Chimeric sequences were identified with UCHIME (Edgar et al., 2011) and removed. Remaining sequences were clustered into OTUs at 97% similarity level using VSEARCH (Rognes et al., 2016) and assigned to taxa using the RDP Classifier with the Silva database (version 132) as reference. All OTUs represented by less than 100 reads were discarded and finally, read counts were resampled to 50438 sequences per sample.

2.5. Network analysis

To evaluate the dependence of community structure on soil texture, and to assess texture specific interactions between species, co-occurrence network analyses were performed for communities along

198 a soil texture gradient. Networks were calculated and analyzed using the molecular ecological network
199 analysis pipeline (MENAP, http://ieg4.rccc.ou.edu/mena/) (Zhou et al., 2011; Deng et al., 2012). The
200 analysis was based on a Spearman rank correlation matrix without log-transformation, calculated from
201 OTUs which occurred in more than 50% of the samples within each texture treatment. In MENAP a
202 threshold of 0.79 was defined, based on random matrix theory. Accordingly, correlation coefficients
203 above 0.79 indicated significantly positive interactions, while correlation coefficients below -0.79 were
204 indicative for negative interactions. The following topological features were calculated: total number
205 of nodes (OTUs), total number of edges, average degree (connectivity), R^2 of power law (describing the
206 proportion of variance explained assuming that the average degree followed a power law function),
207 average clustering coefficient, average path distance, and modularity. Further, among-module
208 connectivity and inter-module connectivity of nodes were calculated, and nodes were assigned to one
209 of the following network roles: network hub, module hub, connector or peripheral. To generate
210 bipartite networks, inter-domain associations between prokaryotes and protists were extracted from
211 full networks. For these networks the connectance, which is the proportion of possible links that are
212 established, was calculated using the Interdomain Ecological Network Analysis Pipeline (IDENAP,
213 http://mem.rcees.ac.cn:8081) (Feng et al. 2022). All networks were visualized in Cytoscape 3.7.2
214 (Shannon et al., 2003).

215 ## 2.6. Statistical analysis

216 Statistical analyses were performed in R version 4.0.3 (R Core Team 2020). The packages 'dplyr'
217 (Wickham et al. 2021), 'ggplot2' (Wickham 2016) and 'RColorBrewer' (Neuwirth 2014) were used for
218 data manipulation and visualization. Treatment differences were compared by analysis of variance
219 (ANOVA) and Tukey's Honestly Significant Difference (HSD) post-hoc test using the package 'agricolae'
220 (de Mendiburu 2021). To test if specific root diameter classes responded differently to the soil texture
221 gradient, roots were grouped into classes of 0-0.5 mm, 0.5-1 mm, 1-1.5 mm and 1.5-2 mm and 2-2.5
222 mm diameter. The proportion of root length assigned to each diameter class was compared between
223 soil texture treatments. The relationship between axial root length and bulk density was analyzed by
224 linear regression.

225 Individual rarefaction analyses were calculated from sequencing data with the package 'iNEXT' (Chao
226 et al. 2014, Hsieh et al. 2020) to assess sufficient coverage of sequencing depth. For downstream
227 analysis of sequencing data, the total number of reads was transformed into relative abundances per
228 sample. After removing two outliers from the prokaryote and three from the protist dataset, OTU
229 richness, Pielou evenness and Shannon diversity were compared by ANOVA, followed by Tukey's HSD
230 or Games-Howell post-hoc test of the package 'rstatix' (Kassambara 2021). The rhizosphere selectivity,

giving the percentual difference between OTU richness in rhizosphere and bulk soil, was calculated for each soil texture treatment according to formula (a):

$$Rhizosphere\ Selectivity\ [\%] = 100 - \frac{OTU\ richness\ rhizosphere\ \times\ 100}{OTU\ richness\ bulk\ soil}$$

Values closer to 0 indicate high similarity between numbers of rhizosphere taxa relative to bulk soil taxa (i.e. low selectivity), while values closer to 100 indicate that a decreasing proportion of rhizosphere taxa was selected from the pool of bulk soil taxa (i.e. high selectivity). The relationship between rhizosphere selectivity and soil texture was analyzed by linear regression. Differences in prokaryote and protist community composition across all treatments were analyzed by Non-Metric Multidimensional Scaling (NMDS) based on Bray-Curtis dissimilarities (relative abundance OTU matrix) and Permutational Multivariate Analysis of Variance (PERMANOVA, 999 permutations) using the package 'vegan' (Oksanen et al. 2020).

3. Results

3.1. Variation in root architecture along the soil texture gradient

A gradient of decreasing bulk density with increasing loam content was successfully achieved, ranging from 1.58 ± 0.022 to 1.372 ± 0.091 g dry wt cm^{-3} in treatments with 20 to 100% loam content, respectively (Table S1). The soil texture gradient had no effect on shoot biomass of maize (Fig. 2a, Table 1), but strongly affected root morphology (Fig. 2c, d, e, f, Table 1). In particular the length of the axial roots (i.e., primary and seminal roots) decreased linearly with decreasing loam fraction and thereby increasing bulk density (R^2= 0.47, $F_{1,35}$= 33.5, p< 0.0001, Fig. 3). At the same time, however, the growth of the lateral roots increased, as reflected in a significant decrease in the ratio between the length of the lateral roots and the length of the axial roots with decreasing loam content ($F_{4,31}$= 12.5, p<0.0001), indicating compensatory root growth. Changes in root length were accompanied by changes in root diameter (Fig. 2d, f). While the proportion of thicker roots with diameters of 1.5-2 mm and 2-2.5 mm was highest in soils with 20, 40 or 60% loam (Fig. 2d, Table 1), the proportion of smaller diameter roots (0.5-1 mm) was increased in soils with 80 and 100% loam (Fig. 2f, Table 1). However, average total root length and surface area did not differ between root systems along the texture gradient (Table 1, Fig. 2b).

3.2. Microbial diversity

After sequence processing 3355 prokaryote OTUs, represented by 9483477 reads and 699 protistan OTUs represented by 5817251 reads were obtained. Rarefaction curves clearly confirmed that the sampling effort was sufficient to cover the whole OTU richness (Supplementary Fig. S1).

In bulk soil, differences in soil texture caused only relatively small changes in the prokaryote and protist diversity, but with generally stronger effects on protists. In prokaryotes, OTU-richness was 10% lower

in the 20% loam treatment than at higher loam contents and quite variable among replicates. Evenness in contrast was slightly (3%) enhanced compared to 100% loam, but Shannon diversity of prokaryotes did not differ between soil texture levels (Fig. 4a, Table 2). Differences of protist OTU richness in bulk soil matched prokaryote richness with lowest OTU richness at 20% loam (Fig. 4b, Table 2). In contrast to prokaryotes, also Shannon diversity and Pielou evenness of protists were lowest at 20 and 40% loam, but decreased again at 100% loam (Fig. 4b, Table 2). The effects of soil texture on alpha diversity were amplified in the rhizosphere. Especially prokaryote OTU richness, Shannon diversity and evenness steeply increased with increasing loam content, again with high variation at 20% loam (Fig. 4c, Table 2). Patterns of protistan alpha diversity in the rhizosphere appeared to follow those observed for prokaryotes, but statistically only 20% loam had significantly lower protistan OTU richness, Shannon diversity and evenness compared to the other soil texture levels (Fig. 4d, Table 2).

In contrast to protists, rhizosphere selectivity on prokaryotes converged towards 0% at highest loam content, indicating the absence of a selective rhizosphere effect on OTU richness, and selectivity increased with decreasing loam content and increasing bulk density (Fig. 5a). At the same time, variability of rhizosphere selectivity of protists and prokaryotes increased. Further, differences of relative read abundances of prokaryote phyla between bulk and rhizosphere soil were much more pronounced in 20% compared to 100% loam (Fig. 6, S2a), indicating a stronger selection of taxa from the bulk soil into the rhizosphere community in soil with low loam proportions. In contrast, the proportion of protistan OTU richness between bulk and rhizosphere soil remained almost constant, showing only higher variability in 20% loam (Fig. 5b). The composition of protistan communities at the order level revealed striking differences between bulk and rhizosphere soil (Supplementary Fig. S2). In both, 20% loam and 100% loam, protists in bulk soil were dominated by the mostly bacterivore Thaumatomonadida while in the rhizosphere soil bacterivore and eukaryvore Glissomonadida, and bacterivore and omnivore Cercomonadida were most abundant.

Generally, community structure of soil microbiota was much less variable in bulk soil than in the rhizosphere. Prokaryote beta diversity clearly shifted according to the soil texture gradient in both, bulk soil and rhizosphere (Fig. 6a, PERMANOVA, bulk soil: $F_{4,35}$=4.3, R^2=0.33, p=0.001; rhizosphere: $F_{4,110}$=12.07, R^2=0.31, p=0.001). Shifts in beta diversity according to changes in soil texture were most clearly pronounced in the rhizosphere where it was coupled with strongly increased variability with decreasing loam content (Fig. 6). Patterns of protistan beta diversity in general followed the patterns of their prokaryote prey, although showing overall less pronounced shifts of community composition in the rhizosphere (Fig. 6b, PERMANOVA, bulk soil: $F_{4,35}$=5.1, R^2=0.37, p=0.001; rhizosphere: $F_{4,113}$=11.75, R^2=0.29, p=0.001).

3.3. Network analysis

Co-occurence networks differd significantly from randomly generated networks (Table 3, Fig. 7). Overall, connectivity of networks followed a power law, but network topologies at loam contents of 20% and 100% differed in characteristic ways. Most strikingly, networks at 20% and 100% loam showed the highest and the lowest clustering coefficients, and modularity was lowest in 20% and highest in 80% and 100% loam, respectively. Due to the decreased OTU richness in 20% loam, numbers of nodes were 43% lower as compared to 100% loam, but these nodes on average had high connectivity (average degree). Overall, increasing the loam content had a strong effect on network connectivity as the numbers of edges dropped by half from 20% to 80% loam, but doubled again at 100% loam. As OTU-richness was increased at high loam contents, average path distance, a measure of distance between nodes and indicative of network size, also increased as expected (Table 3). Accordingly, networks with 20% loam (i.e., 80% sand) were highly clustered and despite containing much fewer nodes and fewer modules as compared to 100% loam, the nodes were highly connected by co-correlating edges. Networks in 100% loam on the other hand were much more modular with many nodes, and only in the 100% loam treatment level numbers of edges (i.e. links) reached a similarly high value as in 20% loam.

Bipartite networks between prokaryotes and protists (Supplementary Fig. S3) were mostly characterized by negative co-occurrences (Table S2). Connectance was highest in 20% loam (0.077), indicative for strongly (mostly negative) correlating prokaryote and protist taxa, but lowest at 100% loam content (0.017).

The composition of bacterial taxa which acted as module hubs differed strongly between the 20% and the 100% loam network (Supplementary Fig. S4). In 20% loam module hubs were represented by Acidobacteria, Actinobacteria, Gemmatimonadetes, Nitrospirae, Proteobacteria and Thaumarchaeota, whereas in the more modular network in 100% loam Bacteriodetes, Chloroflexi, Firmicutes, Planctomycetes, Verrucomicroboa and also three cercozoan taxa held central positions. A network hub was solely found in the 20% loam network and was represented by a Proteobacterium. Exclusively bacteria taxa acted as connectors, mainly in the comparatively complex network in 20% loam. Next to Proteobacteria, Acidobacteria had central positions as module hubs (together with Actinobacteria in all treatments, except 100% loam) and connectors.

4. Discussion

The stepwise manipulation of soil texture led to gradual alterations of root system architecture of maize as hypothesized. Thickening is a typical response of maize roots to higher mechanical impedance (Lynch et al., 2021; Vanhees et al., 2021). More importantly for microbiome assembly, the axial root

length decreased, while the length of lateral roots increased with increasing sand fraction and bulk density, so that average total root length remained constant. This is clear evidence of compensatory root growth at the root system scale (Iijima et al., 1991) and was further supported by similar aboveground biomass production at harvest in our experiment. Sampling bulk soil and rhizosphere allowed a separation of soil texture effects from rhizosphere effects on microbial communities. High variability of OTU richness in bulk soil of 20% loam with a simultaneous decrease in richness of both prokaryotes and protists might be caused by a high and potentially not completely even dilution of loam by quartz sand. However Postma *et al.* (1990) also reported higher variation of inoculated *Rhizobium* sp. together with lower population densities in sand as compared to loam. Also Whitman *et al.* (2018) demonstrated different colonization rates of bacteria and differences in community composition on surfaces of added minerals in the rhizosphere of *Avena barbata*, so that part of the increased variability in coarse-textured treatments might be also related to direct effects of soil texture on community assembly. Alternatively, dilution-to-extinction as an explanation can be excluded as the soil mixture with the highest sand fraction still contained 20% of loam that equals 280 g soil dry wt as inoculum. In addition, we applied conservative filtering on sequences, removing OTUs represented by less than 100 reads for prokaryotes and less than 500 reads for protists, therefore OTU richness is not expected to be affected by missing out rare taxa due to dilution. It is much more likely that the different surface properties of loam and sand favored different bacterial communities (Tanuwidjaja et al. 2021).

Interestingly, the response of protists to differences in texture of bulk soil was more pronounced than for bacteria, showing that these larger microbiota were more strongly affected than bacteria and archaea. Especially the slight, but significantly reduced evenness and Shannon diversity of protists in 100% loam indicates that reduced pore space in fine textured soils may act as a significant environmental filter for the bacterial predators.

In the rhizosphere, effects of soil texture on microbial communities were strongly amplified as compared to bulk soil, especially in prokaryotes. For example, measures of alpha diversity of prokaryotes (OTU richness, Shannon diversity and evenness, Fig. 4c) gradually increased with increasing loam content, while protists quickly reached a maximum alpha diversity (Fig. 4d). Most strikingly, the steeply decreased selection of prokaryote taxa from bulk soil into the rhizosphere with increasing loam content demonstrates a strong effect of soil texture on the recruitment of rhizosphere community members. In comparison, recruitment of protists was much less affected by the interaction of rhizosphere and soil texture. The explicit manipulation of soil texture can thus lead to an improved mechanistic understanding of the processes driving the recruitment of 'core microbiomes' from loam and sand which have been characterized for different plant species (Lundberg et al., 2012; Schreiter et al., 2014; Walters et al., 2018; Dumack et al., 2021).

Increased mechanical impedance to root growth was shown to enhance the sloughing of root cap cells (Iijima et al., 2000) and exudation rates (Groleau-Renaud et al. 1998). Accordingly, soil texture, via effects on root mechanical impedance, exerts direct feedbacks on the quality and quantity of resources available for the rhizosphere microbiota. Slower root growth due to increased soil compaction in consequence will increase the time for species interactions on a given root section. If the quantity and quality of rhizodeposition is the driving factor for microbiome assembly, we might expect interactions between microbiota to become stronger due to greater resource supply, leading to less variability in the composition of the rhizosphere microbiome in coarse-textured soils. On the other hand, the ability of bacteria to keep pace with the growth of roots in soil is a key trait being attributed to microbial rhizosphere competence (Lugtenberg and Dekkers, 1999; Kamilova et al., 2005). Niche preemption through priority effects (Fukami 2015, Attia 2022) of these early colonizers was shown to prevent the random establishment of competing bacteria on roots and leaves (Braun-Kiewnick et al., 2000; Lugtenberg et al., 2001). In addition, Watt *et al.* (2003) discovered that slow elongation rates of seminal roots of wheat favored the invasion of the root microbiome by *Pseudomonas* spp. from organic debris in bulk soil with detrimental consequences for crop productivity. Thus, the advantage of being a colonizer of fast growing roots may fade when root growth slows down at higher mechanical impedance, and instead random invasion from bulk soil may gain importance. The significantly increased variability of beta diversity on root sections of coarse soil texture levels (20% and 40% loam) compared to levels with increased loam content, supports the latter assumption.

Additional features of rhizosphere microbiomes in response to soil texture were revealed by network analysis. Rhizosphere microbiomes in coarse textured soils with high sand proportions showed a strongly clustered network topology with high connectivity among microbiota. A coarse-grained soil environment is characterized by larger pore volumes and higher connectivity at water saturation (Anderson & Domsch, 1995; Holden et al., 2011; Vos *et al.*, 2013; Ebrahimi & Or, 2014) and this apparently facilitated movement and exchange between microsites that led to high co-occurrence. High connectance between bacteria and protists in bipartite networks indicates that also the movement of bacterivores was not much restricted and coarse-grained soils provided access to a wide range of potential prey bacteria. High loam content in contrast caused a modular network structure, indicative for the presence of more isolated sub-communities. This was experimentally confirmed in the same loam soil by Szoboszlay and Tebbe (2021), who found high small-scale heterogeneity of bacterial diversity and network structure among individual soil aggregates of 1 to 5 mg fresh weight, but a homogenization of communities when analysing larger aggregates of 25 to 250 mg fresh weight. It corresponds to the low overall variation of prokaryote alpha diversity in bulk soil of our experiment.

Soil texture further determines the habitable pore space of microbial predators (Elliott et al., 1980; Rutherford and Juma, 1992). Increasing soil loam content results in a higher percentage of soil pores with small neck diameters (Killham et al., 1993) and likely explains the reduced evenness of protists in 100% loam mentioned above. Bacteria are largely protected from predation in pores < 6 µm as provided by soils with high loam and clay content (England et al. 1993). Accordingly, adding small amounts of clay to a sandy soil significantly increased bacterial survival through the creation of protective microhabitats (Heijnen et al., 1992). Network analysis still identified three small amoeboflagellates in Cercozoa as module hubs that apparently still proliferated while the restricted habitable pore space of loamy soils overall reduced alpha diversity of protists. The low connectance of bipartite networks from soils with 100% loam could indicate that these protists mainly preyed on a relative small range of prokaryotes, suggesting high interaction strength (Paine, 1980).

5. Conclusions

Soil texture defines important habitat properties for soil microbiota but it also feeds back on root elongation rate and root system architecture. Decreased axial root length in coarse-grained texture levels was compensated for by enhanced lateral root growth. Effects of soil texture on microbiome assembly were amplified in the rhizosphere and differed between prokaryota and their protistan predators. For example, recruitment of prokaryote taxa from bulk soil into the 'core rhizosphere microbiome' increased strongly with increasing loam content. At the same time variability of alpha and beta diversity increased in the coarse textured soils, and was likely linked to reduced root elongation rate rather than increased rhizodeposition. The rhizosphere microbiomes of coarse textured soils showed a strongly clustered network topology with high connectivity among microbiota, while networks in soils with increasing loam content changed to a modular network structure, indicative of small, isolated sub-communities. Bipartite networks between prokaryotes and bacterivore protists showed low connectance with increased loam content, indicative of increased interaction strength.

Statements and Declarations

Data availability statement

Sequencing data have been submitted to the European Nucleotide Archive and are available under the accession number PRJEB52728. Primer and barcode combinations used for sample indexing and root measurement data can be found online at *(link to supplementary material).*

Author contributions

Michael Bonkowski, Lioba Rüger, Kai Feng and Doris Vetterlein conceived the study, planned the experiment and wrote the manuscript. Lioba Rüger performed the experiment and analyzed the data together with Kai Feng and Ye Deng. Bo Sun, Yan Chen and Ruibo Sun provided processed sequencing

data for bacteria and archaea. All authors read and approved the manuscript. Kai Feng and Lioba Rüger contributed equally to this work.

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Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

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729

730 ## Tables

731 **Table 1.** Analysis of variance in shoot biomass and root morphology measures of plants grown at
732 different soil texture levels. Differences of means were compared among soil texture levels. The
733 table contains *F*-valus with nominator, denominator degrees of freedom (*df*) and *p*-values from
734 comparisons by one-way ANOVA.

	ANOVA		
Parameter	***df***	***F***	***p***
Shoot biomass	4, 30	1.5	0.231
Total root length	4, 30	1.1	0.378
Root surface area	4, 30	1.5	0.229
Ratio of lateral to axial root length	4, 30	12.0	< 0.0001 ***
Ratio of lateral to axial root surface area	4, 30	9.6	< 0.0001 ***
Diameter classes			
0-0.5 mm	4, 33	3.5	0.021 *
0.5-1 mm	4, 33	4.5	< 0.01 **
1-1.5 mm	4, 33	4.5	< 0.01 **
1.5-2 mm	4, 33	8.3	< 0.0001 ***
2-2.5 mm	4, 33	1.1	< 0.366

735

736

737 **Table 2.** Analysis of variance in OUT richness, Shannon diversity and Pielou evenness of prokaryota
738 (bacteria and archaea) and protists (Cercozoa and Endomyxa) in bulk soil and rhizosphere.
739 Differences of means were compared among soil texture levels. Asterisks indicate the significance
740 level. The table contains *F*-values with nominator, denominator degrees of freedom (*df*) and *p*-values
741 from comparisons by one-way ANOVA.

		ANOVA		
	Prokaryota	***df***	***F***	***P***
Bulk soil	OTU richness	4, 35	10.81	< 0.001 ***
	Shannon diversity	4, 35	0.611	0.657
	Pielou evenness	4, 35	3.056	< 0.05 *
Rhizosphere	OTU richness	4, 109	54.47	< 0.001 ***
	Shannon diversity	4, 109	59.1	< 0.001 ***
	Pielou evenness	4, 109	38.79	< 0.001 ***
	Protists			
Bulk soil	OTU richness	4, 35	8.019	< 0.001 ***
	Shannon diversity	4, 35	12.29	< 0.001 ***
	Pielou evenness	4, 35	10.43	< 0.001 ***
Rhizosphere	OTU richness	4, 112	61.54	< 0.001 ***
	Shannon diversity	4, 112	36.39	< 0.001 ***
	Pielou evenness	4, 112	29.28	< 0.001 ***

742

743 **Table 3.** Topological features of empirical and random networks of soils containing 20, 40, 60, 80 and
744 100% loam, including inter and intra domain co-occurrences of bacteria, archaea, Cercozoa and
745 Endomyxa.

	Features	20%	40%	60%	80%	100%
Empirical networks	Total number of nodes	511	737	622	526	911
	Total number of edges	885	775	748	441	885
	R2 of power law	0.995	0.987	0.999	1	0.991
	Average degree	3.464	2.103	2.405	1.677	1.943
	Average clustering coefficient	0.161*	0.079*	0.085*	0.076*	0.061*
	Average path distance	5.174*	10.564*	10.002*	4.097*	10.751*
	Modularity	0.700*	0.896*	0.867*	0.965*	0.932*
Random networks[a]	Average clustering coefficient	0.02 ± 0.003	0.003 ± 0.002	0.007 ± 0.003	0.001 ± 0.001	0.002 ± 0.001
	Average path distance	4.226 ± 0.048	6.945 ± 0.191	5.078 ± 0.101	8.096 ± 0.795	7.311 ± 0.228
	Modularity	0.55 ± 0.009	0.801 ± 0.01	0.71 ± 0.01	0.909 ± 0.008	0.831 ± 0.009

[a] Random networks were generated by randomly rewiring all nodes and links 100 times
** Significant difference ($p < 0.001$) of empirical networks compared to random networks, based on one sample Student's t test*

746

Figures

Fig. 1. Experimental set up. Microcosms were filled with five mixtures of sand and 20, 40, 60, 80 or 100% additional loam. Each soil treatment was replicated 16 times; eight plants were used for measurements of root morphology and eight for microbiome analyses.

Fig. 2. (a) shoot dry weight, (b) total root length, (c) the ratio of the length of lateral roots and axial roots (i.e., primary and seminal roots), (d) the ratio of the surface area of lateral roots and axial roots, (e) exemplary scans of primary roots grown in the five different soil mixtures and (f) the root length proportion assigned to specific root diameter classes. Plants were grown in soil containing 20, 40, 60, 80 or 100% loam. For each soil type measurements were replicated six to eight times. Lower-case letters indicate differences between means (a, b, c, d), asterisks indicate differences between treatments of one diameter class (f) calculated by ANOVA and subsequent Tukey-HSD test.

Fig. 3. Linear decrease in the length of axial roots (i.e., primary and seminal roots) with increasing bulk density. The equation of the linear model is y = -175x + 303 where y is the axial root length, and x the bulk density. Colors of points indicate the loam content in the respective sample.

Fig. 4. OTU richness, Shannon entropy and Pielou evenness of (a) prokaryota (bcteria and archaea) and (b) protists (Cercozoa and Endomyxa) in bulk soil (brown), and (c) prokaryota and (d) protists in the rhizosphere (green). Lower-case letters indicate differences between means within each graph calculated by ANOVA and subsequent Tukey-HSD test.

Fig. 5. Correlations between the rhizosphere selectivity and bulk density for (a) prokaryota (bacteria and archaea) and (b) protists (Cercozoa and Endomyxa). The equations of the linear models are (a) y = 76.54x - 98.3 and (b) y = 20.98x - 27.6, where y is the rhizosphere selectivity, and x is the bulk density. The rhizosphere selectivity is a proportional value, giving the percentual difference between the number of OTUs in the rhizosphere and in bulk soil. Increasing values above 0 indicate an increasing impact on OTU richness through selection. The blue dashed line marks the value where OTU richness in rhizosphere soil and bulk soil is equal.

Fig. 6. NMDS plots of Bray-Curtis dissimilarities of (a) prokaryote (bacteria and archaea) and (b) protist (Cercozoa and Endomyxa) communities at 20, 40, 60, 80 and 100% loam content, in bulk soil (circles, brown) and in the rhizosphere (spuares, green). Non-metric multidimensional scaling was performed using k=4 dimensions, while only the two first dimensions were plotted.

Fig. 7. Microbial co-occurrence networks based on correlation analysis of bacteria (yellow points), archaea (red points) and Cercozoa and Endomyxa (blue points). Networks were calculated for (a) 20, (b) 40, (c) 60, (d) 80 and (e) 100% loam content. Green edges indicate positive correlations and pink edges negative ones. The bar plot (f) shows the proportion of positive and negative correlations between bacteria, archaea, Cercozoa and Endomyxa at the five different loam levels.